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# The Spermatogenesis of *Stenobothrus viridulus*; with Special Reference to the Heterotropic Chromosome as a Sex Determinant in Grasshoppers.

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(Plates 1–3.)

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## Introduction.

Since Van Beneden discovered in 1883 that the somatic number of chromosomes is halved in the mature germ-cells of both sexes, the attention of investigators has been turned to the phenomena of the maturation divisions and to the problems to which they give rise. The literature upon spermatogenesis and oogenesis has become very extensive, and it is impossible to discuss here the numerous questions that have arisen during the last few years. I shall therefore touch only upon certain points of controversy, directly concerned with the morphology and function of the chromosomes.

Although the halving of the somatic number of chromosomes is no longer denied, considerable disagreement exists as to the manner in which reduction is effected. In the eumitotic type of maturation, both mitoses are regarded as being equational; but the majority of cytologists uphold the doctrine of pseudomitosis, in which one maturation division is reductional. They have, however, not decided whether this division is the first or second; and in this way the rival theories of Pre-reduction and Post-reduction have arisen.

The researches of vom Rath in 1892–5 upon the spermatogenesis of

*Gryllotalpa* led him to assert that the first maturation division is longitudinal, the second being transverse and reductional ; and McClung's paper upon *Hippiscus* in 1899 has corroborated this view. Further evidence in support of the theory of Post-reduction has since been supplied by the work of Sutton upon *Brachystola magna,* and by the more recent investigations of Nadine Nowlin and Robertson upon *Melanoplus bivittatus* and *Syrbula admirabilis* respectively. On the other hand, de Sinety, in a paper upon the Phasmidæ, has declared that both maturation divisions in the Orthoptera are longitudinal and equational. In 1905 Montgomery, writing on *Syrbula,* and Farmer and Moore, writing on *Periplaneta,* upheld the theory of Pre-reduction ; and this view has since been adopted by Davis in a paper upon the Acrididæ and Locustidæ, and by Gerard in a paper upon *Stenobothrus biguttulus.*

The studies of Sutton upon *Brachystola magna* led to certain discoveries with regard to the chromosomes themselves: firstly, he found that they exhibited a remarkable degree of isolation, for each became enclosed in a distinct vesicle during the telophase of the secondary spermatogonial mitosis. These vesicles fused later at one polar extremity, with one exception to which I shall allude. Since the chromosomes remain in compartments during resolution into spiremes, he has concluded that their individuality is never lost, and that they are morphologically independent units : this phenomenon has been observed by Otte in *Locusta viridissima,* but is apparently confined to a small number of organisms. The nucleus at this stage usually exhibits a long, continuous, and highly convoluted spireme, or even a complete reticulum, formed by the combined resolution of the chromatin filaments ; Gerard describes this condition in *Stenobothrus biguttulus,* in which he finds no trace of separate vesicles.

Sutton further discovered in *Brachystola* that the chromosomes of the spermatogonial complex invariably show certain size and shape relationships, and that, with one exception, they can be arranged in a graduated series of pairs : this has since been corroborated in other types by the work of Baumgartner, Davis, Gerard, McClung, Montgomery, Nowlin, Robertson, the Schreiners, Stevens, and Wilson. He found moreover that these relationships persist in the later spermatocytes, and, since the number of chromatin bodies is halved at this stage, concluded that a conjugation of members of the spermatogonial pairs had occurred during the intervening period. This view is now held by the majority of cytologists ; and Otte says that he has actually witnessed a side to side conjugation of chromosomes in *Locusta.* Bonnevie, Sainmont, Wilson, and von Winiwarter carry the theory even further, for they believe that there is complete fusion of the associated chromosomes during this period of lateral juxtaposition ; on the other hand, the entire theory of conjugation is denied by Duesberg, Fick, Gerard, and Meves.

This theory has been eagerly seized by Mendelians to explain the

segregation of character factors necessary to that mode of inheritance: the members of each spermatogonial pair are assumed to be respectively paternal and maternal in derivation, so that the juxtaposition of their component chromomeres permits the exchange of character factors obtained from the two parents. This is merely an hypothesis, but there seems to be little doubt that the number and size and shape relationships of the chromosomes are constant for the species; and it is probable that we shall eventually find morphological correlation between the complexes of allied members of a group.

Lastly, there is the problem of the heterochromosomes, investigated originally by Wilson, and divided by him into three classes—idiochromosomes, heterotropic chromosomes, and microchromosomes. The first-named consist of two elements, differing in size and staining deeply during the resting stages and growth period of the primary spermatocytes; they later conjugate, and still later divide, the larger passing to one pole and the smaller to the other. The oogonia show a corresponding pair of chromosomes, but in this case both are of the same size. Spermatozoa possessing the larger idiochromosome produce females, those possessing the smaller produce males. The heterotropic chromosome occurs in the spermatogonial cell as a single element, and behaves like the ordinary chromosomes in the second maturation mitosis, but passes entire to one daughter cell at the first. As in the case of the idiochromosome, it is represented in the oogonia by a pair. Spermatozoa containing the heterotropic chromosome produce females, and those without it males. Wilson has suggested that, in the male, it acts as a male determinant, and that it passes from one sex to the other alternatively, being recessive in the female: Hertwig, Paulmier, and Wassilieff regard it as a degenerating chromosome that will eventually become extinct—a view strongly opposed by McClung.

In 1899 McClung drew attention for the first time to this peculiar chromosome in the male germ-cells of *Xiphidium*; and it has since been studied in a large number of organisms, particularly Orthoptera. He found that it undergoes no resolution into a spireme during the primary spermatocyte resting-stage, but persists as a compact and darkly staining body on the periphery of the nucleus: he erroneously stated that it divides longitudinally at both maturation divisions, but corrected this mistake in a later paper upon the Locustidæ. This "accessory" chromosome of McClung has been found by de Sinety in the Phasmidæ, and by Sutton in *Brachystola*: Baumgartner has studied it in *Gryllus*; and his results have been confirmed by Gutherz, working upon the same material. Otte has observed it in *Locusta*, Gerard in *Stenobothrus biguttulus*, Nowlin in *Melanoplus bivittatus*, and Robertson in *Syrbula admirabilis*: Davis has seen it in every member of the Acrididæ and Locustidæ that he has studied, and further, has shown that this "monosome" is represented in the oogonia by a pair of

chromosomes. He found it in certain cases enclosed in a vesicle during the resting-stage, but considers this condition artificial and unimportant.

Somewhat different results were obtained in 1905 by Montgomery working upon *Syrbula acuticornis*, for he declared that the heterotropic chromosome is represented in the spermatogonial cell by two chromosomes, and that it divides at both maturation divisions. Robertson's researches however upon the closely allied *S. admirabilis* afford no evidence of this paired condition, and support the view that this chromosome passes entire to one pole at the first maturation division, splitting longitudinally at the second : this seems to be the normal occurrence in the Orthoptera, for it has been observed by Baumgartner, Davis, Gerard, Gutherz, McClung, Nowlin, Otte, Robertson, de Sinety, Sutton, Wilson, and others.

The discovery in the male germ-cell of an odd chromosome, which passes entire to one pole at a subsequent mitosis, and the discovery that in allied types the unequal members of one spermatogonial pair pass to opposite poles have proved that dimorphism of spermatozoa exists in certain groups : and, since spermatozoa of the one kind produce males, and those of the other females, sex, in these organisms, must be determined at the moment when the spermatozoon enters the micropile, immediately prior to amphimixis. This has given rise to the hypothesis that dimorphism of spermatozoa occurs throughout the animal kingdom, and that sex is determined in this manner.

It is possible that the presence or absence of a particular chromosome is the factor controlling sex ; but it is equally possible that this chromosome contains only certain of the numerous characters peculiar to one sex, and that its passage to one pole is closely connected with the passage to that pole of the ordinary chromosomes, after they have divided on the equatorial plate. The function of the chromosomes is not yet understood : although the majority of cytologists believe that the chromatin alone contains the bearers of the hereditary characters, some still affirm that the cytoplasm is the sole agent in this respect, and that the chromatin fulfils the subordinate rôle of a nutritive substance. The experiments of Boveri upon the fertilization of enucleated Echinoderm ova appeared convincing, but unhappily the same experiments repeated by Delage and others gave diametrically opposite results. It seems of little importance whether the transmitted material is composed of actual character factors, or whether it represents a concatenation of physical units, resulting in the phenomena implied in heredity ; but it is important to ascertain by what means these phenomena are reproduced generation after generation.

The character factors may eventually be found to reside in both chromatin and cytoplasm, being distributed in the latter during the resting-stages for purposes of nutrition, and being collected together in the chromatin filaments only during the stages immediately preparatory to karyokinesis: this would

explain the resolution of the chromosomes into spiremes or reticulum, and their later shortening and consequent closer association of granules—the chromatin in this case serving merely as a convenient vehicle for the precise distribution of character factors, or their equivalents, between the two daughter cells.

## Material and Methods.

My material was collected at Nannerch, in Flintshire, N. Wales, in the last week of August 1909. The grasshoppers were killed in chloroform within a few hours of capture, and were placed whole in the fixative after the wings and legs had been removed, and the integument of the back slit up to allow readier access to the fluid. I have obtained excellent results with Perenyi's chromo-nitric acid solution, the resting-stages and various phases of mitosis being very perfectly preserved : the majority of writers on insect spermatogenesis, however, appear to have used Flemming's strong chromo-aceto-osmic acid solution, Hermann's platino-aceto-osmic acid solution, or the fixatives of Bouin and Zenker.

The grasshoppers were transferred after two hours to a 50 % aqueous solution of alcohol, and an hour later were placed in a 70 % solution, in which they remained for twelve hours ; they were then stored in a solution of 80 % alcohol. This storage solution was changed twice during the first month, having become thick and discoloured with pigment.

When required for embedding, the testes were dissected out, and placed for twenty-four hours in a 90 % solution of alcohol : after being passed through absolute alcohol and cleared in cedar-wood oil, they were embedded in paraffin, remaining for twenty minutes in the first bath and for fifteen in the second. I used paraffin with a melting-point of 52° C., since I found that paraffin with a higher melting-point had a tendency to overheat the cells. Sections were cut with an ordinary Cambridge rocking microtome to thicknesses varying from 5 to 10 $\mu$, and were invariably stained on the slide. The nuclear stains used were Heidenhain's iron hæmatoxylin, iron brazilin, and safranin, the first-named being used alone or in conjunction with a plasma stain—*e. g.*, eosin, congo-red, or picro-carmine ; I also used the tricolor stain of Flemming, and the permanganate of potassium method of Henneguy.

In staining with the iron hæmatoxylin, I used, as a mordant, an aqueous solution of iron alum, in which the slides remained for six hours ; they were then stained for twelve or fifteen. Davis left his slides in the mordant for only two hours, and in the stain for from four to six ; but I have found that the longer period gives better results as regards sharp definition, while the process of differentiation can be more perfectly controlled. In the cases where a second stain was used, the slides were left for ten minutes in the plasma stain before being transferred to the iron hæmatoxylin : the iron

alum has no effect upon the former, but this cannot be said of the alcohol; so great care must be taken not to wash out the whole of the plasma stain in the subsequent process of dehydration through successive strengths of alcohol. The iron hæmatoxylin gives the best results in all cases where it is required to bring the chromosomes and nucleoli into evidence; and this is particularly noticeable when camera-lucida drawings are needed. Davis obtained his best results with iron hæmatoxylin in conjunction with bordeaux-red, and has confined himself almost entirely to this combination.

When staining with safranin I used a 50 % solution in alcohol, leaving the slides in it for from twelve to twenty-four hours; this gives an orange-grey tint to the protoplasm, the chromatin staining bright red. Henneguy's method is a modification of this, for the safranin used is made by Zwaardemaker's formula, being a mixture of equal volumes of alcoholic safranin and anilin water: the slides were placed for five minutes in a 5 % aqueous solution of permanganate of potassium, which acts as a mordant, and then stained for six or twelve hours, after careful washing in running water. The excess of colour was removed by a high strength of alcohol. Wilcox used this method when working upon *Caloptenus femur-rubrum*, and obtained good results; he however allowed the slides to remain in the stain only for a few minutes.

In the iron brazilin method, first described by Hickson *, no second stain is necessary, for the cytoplasm as well as the chromatin is affected: the slides were placed for two or three hours in a solution of iron alum in 70 % alcohol, and were then stained for from sixteen to twenty-four hours. This stain is useful for studying late stages of unripe spermatozoa and their earlier spermatid transformations.

The tricolor stain of Flemming gives very delicate results, particularly in stages other than those of actual mitosis. I stained in safranin for forty-eight hours, and washed the superfluous colour out with strong alcohol; the slides were then taken down to water through successive strengths of alcohol, and were stained for several hours in an aqueous solution of gentian, after which they were washed in water and placed for ten minutes in a similar solution of orange G, which acts as a differentiating agent for the gentian. This combination gives a purple tint to the chromosomes and nucleoli, the spindle fibres, &c., appearing in various shades of grey and brown.

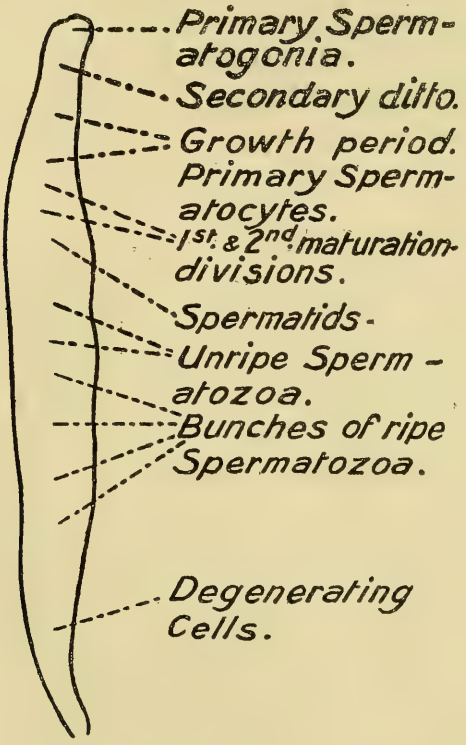
## The Follicles of the Testis.

The testes are two ovoid paired organs lying dorsally to the alimentary canal in the middle of the abdomen, and so closely associated that they can

* Hickson, S. J., "Staining with Brazilin," Quart. Journ. Micr. Sci. xliv. p. 469, 1901.

be dissected out as a single body: they consist of a number of tubular follicles, tapering at the ends, and opening posteriorly into a duct communicating with the vas deferens.

By numbering the follicles in a section, and allotting the same numbers to corresponding follicles in successive sections of a complete series, it is possible to reconstruct the follicle in any particular case, and to recognize the true position of one section in the whole, in cases where the razor has cut transversely or at an angle to the plane of length.




At the anterior end of each follicle is a single cluster of primary spermatogonia with the apical cell, and several clusters of secondary spermatogonia, arranged without definite order. The resting- or growth-stages of the primary spermatocytes occupy a considerable area, lying posteriorly to the spermatogonia; and the heterotropic chromosome is here seen for the first time as a dark and compact body apposed to the nuclear membrane.

We next see the various phases of the primary and secondary spermatocyte mitoses, there being no resting-stage between these two divisions. Proceeding still further towards the posterior end of the follicle, we meet with the transformation from spermatids to unripe spermatozoa; the former are

in scattered groups, and the latter in more closely associated bunches. Beyond these are dense masses of ripe spermatozoa, placed at considerable intervals in the lumen of the follicle: the extreme end is occupied by degenerating cells that will undergo no further development.

The posterior half of the follicle is occupied by unripe and ripe spermatozoa, and the greater part of the anterior half is closely packed with the primary spermatocyte growth-stages. The follicle is divided into tracts, in which these various stages are found, the partitions arising from the follicle wall: further subdivision is effected by septa, dividing the tracts into cysts. Cells in one cyst are not all at the same stage; and the precocious cells of one section correspond with the laggards in the next. When the follicle has been cut at right angles to its length, the succession of stages can be followed with great accuracy until we come to the spermatids, when the identity of the follicle is lost, clusters of spermatozoa alone being distinguishable.

## Spermatogonia.

The extreme anterior zone of the mature follicles is divisible into two parts, occupied respectively by the primary and secondary spermatogonia. The former are arranged in a single layer round a central cell—the apical cell—recognizable by its regularly ovoid nucleus, in which lies a group of large and deeply staining granules, the ordinary chromatin particles being distributed in irregular blotches. The nuclei of the primary spermatogonia are situate in the region of their cytoplasm furthest from the apical cell, and present a lobulate appearance, as can be seen in fig. 1, on Plate 1. The chromatin is disposed in minute particles upon the linin threads of an apparently complete reticulum; and I have failed to find any massing of larger granules, as in the case of the apical cell. Each follicle contains one apical cell with its attendant primary spermatogonia.

At present little is known of the nature of this apical cell, which has been found and studied in many insect forms, but principally in the Lepidoptera; its function is not yet understood, but it probably plays an important rôle by affording either nourishment or physical support to the cells destined to become spermatozoa. On the other hand, it has been suggested that it is a degenerate spermatogonial cell, or the mother cell of the primary spermatogonia surrounding it, or that its function is connected with the formation of the zones into which the follicle portions are subdivided. Davis has found it in the members of the Acrididæ and Locustidæ that he has studied, and has shown that in *Dissosteira carolina*, *Arphia tenebrosa*, *Chortophaga viridifasciata*, and *Stenobothrus curtipennis* it is completely surrounded by the single layer of primary spermatogonial cells, but only partly surrounded in *Melanoplus femoratus* and *Hippiscus tuberculatus*, being at one side in

contact with the connective-tissue cells. Gerard further distinguished it in *Stenobothrus biguttulus* by its deeper cytoplasmic colouring ; but I have not found this in my material. The cluster of primary spermatogonia is surrounded by numerous connective-tissue cells, recognizable by their small size and deeply stained nuclei. The secondary spermatogonial groups are completely enclosed by cyst-walls, formed from connective-tissue cells, and lie posteriorly to the primary spermatogonia in irregularly disposed clusters, often so closely packed that the cells become distorted. Each group has been formed by repeated division of a single cell, originally extruded from the primary spermatogonial figure, so that there is a continuous stream of cells passing towards the posterior end of the follicle. A secondary spermatogonial cyst is shown on Plate 1. fig. 2, from which it will be seen that these cells closely resemble the primary spermatogonia, but are easily distinguishable by the absence of the apical cell.

As in the case of the primary spermatogonia, the resting-stage nucleus shows a series of chromatin granules disposed along linin threads : I have been unable to discover whether we are dealing here with one continuous thread, much convoluted, or with a number of threads, irregularly placed in such a manner that they combine to give this reticular appearance. Montgomery seems to have experienced the same doubt in the case of *Syrbula*. Davis merely describes a network, in which chromatin granules are massed at the intersections of the linin threads.

There are two spermatogonial generations ; and this agrees with the results of Montgomery upon *Syrbula acuticornis*, Gerard upon *Stenobothrus biguttulus*, and Davis upon numerous members of the Acrididæ and Locustidæ. Sutton however has declared that there are eight in *Brachystola* ; and Wilcox has been unable to determine the exact number in *Caloptenus femur-rubrum*. McClung suggests that the number varies with the species.

In the resting-stages the nucleus is not deeply stained. The prophase of division is characterized by the flowing together of granules on the linin threads ; and these chromatin particles become more and more closely associated until they form the ragged filaments, representing the forerunners of the compact bodies that later appear on the periphery of the karyokinetic spindle. As condensation proceeds, the chromatin exhibits greater affinity for the iron hæmatoxylin, so that distinct correlation exists between the intensity of staining and the degree of proximity of the associating particles. The formation of the spindle is preceded by the appearance of two small asters in the cytoplasm, close to the periphery of the nucleus. The chromatin filaments have by this time assumed the shapes and sizes characteristic of the chromosomes ; and they now arrange themselves on the equatorial plate, preparatory to division, the metaphase complex showing seventeen chromosomes of varying shapes and sizes.

In every complex that I have studied I have found the same number of chromosomes, and the same size and shape relationships. With the exception of the heterotropic chromosome, the members of the complex can be arranged in a graduated series of eight pairs, divisible into three groups, small, large, and medium: there are three small pairs, of which two are spherical and the third ovoid, three pairs of large rod-shaped chromosomes bent slightly at the middle, and two pairs of medium chromosomes, which usually appear as straight rods. The heterotropic chromosome is the fourth largest in the complex, and is a straight or very slightly bent rod, recognizable for the first time at this stage. By choosing metaphases where the chromosomes overlap only to a small extent, and by making camera-lucida drawings upon successive occasions and comparing results later, I have tried to minimize the possibility of error in counting the number of chromosomes present; this difficulty is not experienced in the metaphases of the spermatocytes, where only half the spermatogonial number is found, and where cells can be chosen in which no overlapping occurs. Plate 1. figs. 3 & 4 show polar views of the spermatogonial metaphase, the seventeen chromosomes being arranged on the equatorial plate.

Gerard has found seventeen chromosomes in the spermatogonial complex of *Stenobothrus biguttulus*, and Davis's results in the case of *S. curtipennis* agree with this. McClung in an early paper suggested that the number is dependent on the family, and is a constant, but this has not been found to be strictly true. I believe the number is constant for the genus, but not for a larger subdivision of the animal kingdom. Since the number has been found to vary in the Orthoptera, it is interesting to compare the results of writers upon this subject. Sutton has found twenty-three chromosomes in *Brachystola magna*, capable of being arranged in three small and eight large pairs, with an odd or heterotropic chromosome ranking among the latter. Davis has counted the same number in *Arphia tenebrosa, Hippiscus tuberculatus, Chortophaga viridifasciata*, and *Melanoplus femoratus*. In the Locustid, *Steiroxys trilineata*, he has found twenty-nine, and has shown that in all cases the ordinary chromosomes can be arranged in pairs forming a graduated series. McClung has observed thirty-three chromosomes in *Xiphidium fasciatum*; and Nadine Nowlin has counted twenty-three in *Melanoplus bivittatus*. When working upon crickets, Baumgartner found twenty-nine in *Gryllus assimilis* and twenty-one in *G. domesticus*.

The chromosomes, after placing themselves on the spindle, divide longitudinally, and their halves pass to opposite poles; the division of the heterotropic chromosome is longitudinal, but occurs often at a later stage, when the ordinary chromosomes have begun to move apart: an example of the secondary spermatogonial telophase in shown on Plate 1. fig. 5. On reaching the poles the chromosomes elongate and appear to lose their affinity for the iron hæmatoxylin: as the nuclear membrane reforms, they

become more and more ragged ; and this dissociation of chromatin continues until we see again the characteristic resting-stage with its chromatin granules disposed along linin threads, which combine either in reality or in appearance to produce a complete reticulum. The whole process is merely an inverse repetition of that preceding division. The heterotropic chromosome takes no part in this diffusion of chromatin, and remains throughout this stage as a darkly stained and homogeneous body apposed to the nuclear membrane, where its affinity for the stain and smooth outline render it extremely conspicuous.

### Primary Spermatocytes.

After the last spermatogonial division, resulting in the formation of two daughter primary spermatocytes, the nucleus is much reduced in size. McClung has pointed out that at this stage reproduction is replaced by constructive metabolism, and that the chromosomes, after exhausting their metabolic resources, unite their common energies to build up a new cytoplasm. This suggestion probably furnishes the true explanation, but in any case possesses considerable pragmatic value, for some process of this nature undoubtedly occurs.

The cells undergoing this resting- or growth-stage occupy large areas in the follicle, and the gradual increase in size as we proceed more and more posteriorly is very noticeable. This growth-period is continued until the nucleus has attained its maximum size, when the cell enters the prophase of the next mitosis. Plate **1.** figs. 6 & 7 show the difference between the primary spermatocyte immediately after the secondary spermatogonial division and immediately before the next mitosis. The nucleus shows a reticulum, composed of chromatin granules placed along linin threads, the individuality of the ordinary chromosomes being completely lost.

It will be remembered that at this stage Sutton found no loss of individuality of the chromosomes in *Brachystola* ; each chromosome underwent resolution into a spireme in a separate sac, in which it remained completely isolated, although the sacs fused at one end to form a common chamber. He consequently met with no reticulum, or appearance of a reticulum, and so put forward this phenomenon as a convincing proof of the individuality of the chromosomes. Robertson observed a similar condition in *Syrbula*, but did not always find the sacs, containing the ordinary chromosomes, clearly distinguishable. Both Sutton and Robertson describe a distinct vesicle, in which the heterotropic chromosome lies, having no morphological connection with the vesicles of the other chromosomes. I have been unable to find the smallest trace of such vesicles, and am confirmed in this by the work of Gerard on *Stenobothrus biguttulus*—a member of the same genus.

The prophase of division is characterized by the closer association of chromatin granules on the linin threads of the reticulum; and this process continues until the latter is resolved into a number of ragged filaments, which shorten and thicken, and later assume a boomerang shape. By this time all trace of the component granules is lost; and the ragged horseshoe bodies, folding themselves into figures of eight and rings, are gradually transformed into the smooth and clearly defined chromosomes. The resolution of the reticulum into filaments is shown on Plate **1.** fig. 8, and the subsequent shortening and thickening of the boomerangs in fig. 9 of the same Plate. The various shapes assumed by the chromatin filaments at a still later stage are shown on Plate **2.** figs. 10–19, the most prominent types being crosses, rings, and loops. The last-named may be doubled to form a complete figure of eight, or may form a single loop with free ends twisted or crossed over one another.

As soon as the centrosomes have taken up their position at the poles, the chromosomes appear on the equatorial plate, and the characteristic metaphase figure is once more represented. The heterotropic chromosome remains as a dark and smoothly outlined body close to the nuclear wall while the chromatin filaments are being transformed into chromosomes: it then takes its place among them on the mitotic spindle. The number of filaments evolved from the reticulum is eight, so that nine chromatin bodies compose the metaphase complex. In this manner the sixteen ordinary chromosomes of the spermatogonial cell have been halved, and this reduction must be effected before the breaking up of the spireme, for I have found no evidence of lateral association of filaments after this has occurred. Gerard has obtained similar results, but explains the reduction of the somatic number by describing an association of granules, the reticulum meshes combining in pairs by means of fine anastomosing threads; he has found that this process always begins near the heterotropic chromosome. On the other hand, Davis denies that a continuous spireme is formed, but mentions a similar massing of granules at this stage; he describes how the spiremes appear later in the form of loops, attached by their free ends to the nuclear membrane, and, since the number of loops is half the somatic number of the chromosomes, suggests that each loop is composed of two univalent chromosomes united end to end.

The chromosomes on the equatorial plate exhibit the same size and shape relationships found in the spermatogonial complex: of the eight ordinary chromosomes, three are large, three small, and two of medium size, the heterotropic chromosome again being the fourth largest. This can be seen from Plate **2**, where a polar view of the metaphase is given in fig. 20. The smaller chromosomes are the first to divide, division in all cases being longitudinal; this agrees with the results of Baumgartner, Gerard, Henderson, McClung, Montgomery, Nowlin, Robertson, Sutton, Wilcox,

and others. The heterotropic chromosome does not divide at this mitosis, and may often be seen on its way to one pole, while the ordinary chromosomes are still on the equatorial plate. Illustrations of this are shown on Plate 2. figs. 21 & 22, and a still later stage in fig. 23, where the ordinary chromosomes, still attached to one another by connecting-fibrils, are moving towards the opposite poles. In every case the heterotropic chromosome passes entire to one daughter cell, so that dimorphism of the spermatozoa is effected at this mitosis, half the resulting secondary spermatocytes possessing this odd chromosome, and half being without it. No resting-stage follows the telophase, for the two maturation divisions occur in rapid succession.

### Secondary Spermatocytes.

Large areas are frequently to be seen occupied by cells undergoing these two divisions; and the absence of an intervening resting-stage is not characteristic only of this species, for it has been observed in many other types.

The chromosomes that assemble on the mitotic spindle are nine or eight in number, the difference depending upon the presence or absence of the heterotropic chromosome, which is found in only half the cells. The complex exhibits the same size relationship that occurred in the earlier metaphases: there are once more three small chromosomes, of which two are spherical and the third ovoid, three large chromosomes appearing as V's with their component arms closely folded on one another, and two chromosomes of intermediate size, also represented by a pair of arms joined at one extremity. The heterotropic chromosome, when present, is still the fourth largest of the complex. Examples of polar views of the metaphase, showing the chromosome complex, are given on Plate 2. figs. 25, 26, & 27.

Later we see the ordinary chromosomes dividing in mitosis at the junction of their component arms, one arm going to each pole. If the arms are really the associated members of the spermatogonial pairs, this division must effect the separation of chromosomes that became laterally associated two generations previously; and if we accept the further hypothesis that the members of these pairs are respectively paternal and maternal in derivation, we must regard this mitosis as the means of separating chromosomes obtained from the two parents, after a possible exchange of chromatin. There is however no direct evidence to prove that the component arms of these V-shaped chromosomes correspond with the members of the spermatogonial pairs, or that the members of the pairs are derived from both parents, although there are reasons for assuming the truth of these two suppositions.

The heterotropic chromosome is the last to divide, and can often be seen on the periphery of the mitotic spindle when the ordinary chromosomes are

massing at the poles ; on these occasions it appears in the form of two V's, whose apices are distally placed with respect to one another. An illustration of this phenomenon is given on Plate **3.** fig. 28. This "lagging" of the heterotropic chromosome has been observed by Baumgartner in *Gryllus*, by Davis in numerous members of the Acrididæ and Locustidæ, and by many writers in the case of other types.

The cytoplasm becomes constricted shortly after the chromosomes have passed to the two poles, and in this manner two daughter spermatids are formed.

### Spermatids.

As soon as the nuclear membrane of the spermatids has formed, the chromosomes become ragged, and dissociation of their component granules begins to take place. This process continues until the individuality of the ordinary chromosomes is completely lost, the nucleus exhibiting several irregularly placed blotches, in which the chromatin granules are faintly distinguishable. The heterotropic chromosome retains its individuality for a considerable time, remaining intact as a darkly stained body in the midst of the dissociating ordinary chromosomes ; it subsequently becomes resolved into a mass of granules, and loses its identity in the general chromatin reticulum. Plate **3.** fig. 29 shows a cell at this stage of development.

Shortly after this a body appears outside and in contact with the nuclear membrane, forming a conspicuous object on account of its great affinity for the iron hæmatoxylin and its characteristic bead or knob shape. The axial filament grows out from a clearly marked constriction in the middle of this "centrosome," and, as the cytoplasm of this region gradually elongates to form the tailpiece of the unripe spermatozoon, increases in length and appears as an indistinct line down its entire length. The chromatin granules have now become extremely minute, and the nucleus has the appearance of an uniformly grey body, in which the component chromatin particles cannot be distinguished. This gradual transformation is shown on Plate **3.** figs. 30 & 31.

The elongation of the tail and axial filament is later accompanied by a corresponding elongation of the nucleus, which loses its spherical shape and appears in that of a torpedo. The "centrosome" still occupies its original position between the axial filament and the posterior end of the nucleus, but is slightly reduced in size ; this can be clearly seen in figs. 32 & 33. The lengthening of the nucleus and tailpiece continues, and the "centrosome" becomes smaller and smaller. The nucleus gradually becomes darker, doubtless owing to a closer association of its chromatin particles. Plate **3.** figs. 34 & 35 show this further elongation, the reduced size of the "centrosome" being very noticeable.

The torpedo-shaped spermatids are to be seen scattered in the follicle, and are very prominent objects on account of the strong staining of the iron hæmatoxylin : they are placed usually with their heads towards the anterior end of the follicle, and travel as unripe spermatozoa, tail first, towards the posterior end. As they elongate further into the thin thread-like form characteristic of the unripe spermatozoa, they become associated in ragged clusters, which continue to condense until we find solid masses of ripe and finely drawn out spermatozoa situate at irregular intervals in the lumen of the follicle. Plate 3. fig. 36 shows the penultimate stage immediately preceding the transformation into the mature spermatozoon, and is a good example of the much reduced "centrosome" and the darkly staining and considerably elongated nucleus.

## Summary.

1. The apical cell is found at the extreme anterior end of the mature follicle, completely surrounded by a single layer of primary spermatogonial cells. There is only one apical cell in each follicle.

2. The secondary spermatogonia occur in clusters and morphologically appear similar to the primary cells, but are recognizable by the absence of the apical cell and by the number of clusters.

3. The nucleus exhibits an apparently complete reticulum in the resting-stages of both primary and secondary spermatogonia : there is no trace of the identity of either heterotropic or ordinary chromosomes.

4. The chromosomes of the spermatogonial complex can be arranged in a graduated series of pairs, and are divisible into three groups, viz., large, small, and medium-sized chromosomes. The number of chromosomes is constant and is seventeen, the fourth largest being unpaired and corresponding with the "monosome" and "accessory" chromosome of other writers.

5. All the members of the spermatogonial complex divide in mitosis ; but the odd or heterotropic chromosome often "lags," and can be seen on the spindle when the ordinary chromosomes are assembling at the poles.

6. The nucleus is at its smallest size after the last spermatogonial division, and this stage is followed by a clearly observable growth-period, extending to the prophase of the first maturation division. In this resting-stage the nucleus again exhibits a chromatin reticulum, the granules being disposed along linin threads : the identity of the ordinary chromosomes is lost, but the heterotropic chromosome remains as a dark and homogeneous body close to the periphery of the nucleus, and undergoes no resolution into a spireme.

7. I have found no trace of separate sacs or vesicles in which chromosomes undergo transformation into spiremes, either in the case of the heterotropic or the ordinary chromosomes.

8. The equatorial plate of the primary spermatocyte mitosis shows nine

chromosomes, again divisible into three groups as regards size. The fourth largest of the complex is undoubtedly the heterotropic chromosome. The distinct correspondence between the size and shape relationships of the secondary spermatogonial and primary spermatocyte complexes points to the possibility of a lateral conjugation of members of the spermatogonial pairs during the intervening period, but is not a proof of it.

9. The ordinary chromosomes divide in the primary spermatocyte metaphase, and their halves pass to opposite poles of the spindle : the heterotropic chromosome shows no sign of division, and passes entire to one daughter cell, while the ordinary chromosomes are still on the equatorial plate. In this manner dimorphism of the subsequent spermatozoa is effected.

10. I have been unable to discover whether reduction—the separation of conjugant members—occurs at the first maturation division or at the next ; possibly both divisions are equational, and only a numerical reduction takes place as a result of lateral association of chromatin granules or masses on the reticulum threads prior to the primary spermatocyte prophase of mitosis.

11. There is no resting-stage between the first and second maturation divisions ; the constriction of the cytoplasm to form the two daughter secondary spermatocytes is closely followed by the appearance of the next karyokinetic spindle.

12. The complex of the secondary spermatocyte cell shows nine or eight chromosomes, and this difference is due to the presence or absence of the heterotropic chromosome, which is found in only 50 % of the cells. The chromosomes exhibit the same size relationships that occurred in the previous metaphases, there being three large chromosomes, three small, and two of intermediate size. The heterotropic chromosome, when present, is the fourth largest of the complex. Two of the three small chromosomes are spherical, and the third is ovoid or slightly dumb-bell shaped : the remainder appear as two arms jointed at one end and closely apposed to one another.

13. I find no direct evidence to prove that these arms are the representatives of the spermatogonial pairs.

14. Division occurs at the junction of the component arms. The heterotropic chromosome usually "lags," and can be seen on the spindle when the ordinary chromosomes have passed to the two opposite poles.

15. The formation of the nuclear membrane in the spermatids is followed by resolution of the ordinary chromosomes into their component granules. This process continues until the nucleus appears of an uniformly grey colour, in which the individuality of the chromatin particles is lost. The heterotropic chromosome remains at first as a darkly staining and irregular body, but later undergoes resolution into particles, whose identity is indistinguishable in the common chromatin mass.

16. The appearance of the "centrosome" is followed by the formation of

the axial filament, arising near the constriction in the middle of this body. The cytoplasm in this region elongates to form the tail of the unripe spermatozoon, and the axial filament appears as a faint line running down the centre of the tail. The elongation of the nucleus and the tailpiece continues, and is accompanied by a reduction of the "centrosome," which finally becomes extremely small.

17. The head of the spermatozoon is composed of the nucleus; the "centrosome" forms the middle piece, and the axial filament and its surrounding cytoplasm form the tail.

18. The spermatids travel later towards the posterior end of the follicle with their heads turned towards the anterior end; and this phenomenon is observable in the spermatozoa. The spermatids are found scattered in the follicle, the unripe spermatozoa in more closely associated clusters, and the ripe spermatozoa in solid bunches.

19. At no stage have I observed a discharge of chromatin from the nucleus, and I have seen nothing to suggest that the whole of the chromatin is not directly concerned with the transformation from the resting reticulum to the compact chromosome condition of the metaphase.

20. The extreme posterior end of the follicle contains numerous degenerating cells, in which irregularly shaped masses of chromatin stain deeply with the iron hæmatoxylin.

21. Although the individuality of the chromosomes is completely lost in the resting-stages of the spermatogonia, spermatocytes, and spermatids, with the one exception of the heterotropic chromosome in the primary spermatocyte growth-period, there is strong reason for supposing that the same elements appear on the successive mitotic spindles throughout development. It is possible that the component granules of a particular chromosome are not the same in these cases, for an exchange of chromatin particles may occur during the reticulum stages, and if this occurs we have at present no means of discovering the extent of this exchange. It must, therefore, not be assumed that corresponding chromosomes of two successive metaphases contain the same individual chromomeres.

In conclusion, I offer my thanks to Prof. Hickson and his staff for placing the research laboratories of Victoria University at my disposal, and for the encouragement that I have received in carrying out this research.

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The Zoological Laboratories,
Victoria University, Manchester.
October 1910.

## EXPLANATION OF THE PLATES.

The original drawings were made with the aid of a large Abbe camera lucida, and are reproduced here at the same magnification. All the figures are magnified 2500 diameters, except figs. 1 and 2, which are magnified only 750 diameters. The preparations were studied by means of a Zeiss apochromatic oil-immersion objective of 2 mm. focus and N.A. 1·30, and compensating oculars Nos. 6, 12, and 18. Illumination was obtained from an acetylene burner, the light being directed on to the mirror through a globe condenser containing a weak aqueous solution of copper acetate.

All the drawings shown are made from preparations fixed with Perenyi's fluid and stained with Heidenhain's iron hæmatoxylin, and in certain cases counterstained with eosin. The sections were cut to a thickness of 10 $\mu$. I have endeavoured to render comparison between the successive stages easy by adopting this plan of drawing the figures to the same magnification.

### Plate 1.

Fig. 1. Cyst containing apical cell surrounded by a single layer of primary spermatogonial cells. The lobulate appearance of the distally placed nuclei of the latter is very noticeable. The apical cell's nucleus exhibits the irregular masses of chromatin and the peculiar cluster of deeply staining granules.

Fig. 2. Cyst containing a cluster of secondary spermatogonia, and recognizable by the absence of the apical cell.

Fig. 3. Secondary spermatogonial metaphase, seen from one pole. The seventeen chromosomes are paired, and can be divided into three groups as regards size. The heterotropic chromosome is the fourth largest, and is marked **X**.

Fig. 4. Ditto.

Fig. 5. Lateral view of secondary spermatogonial telophase. The heterotropic chromosome has divided late.

Fig. 6. Resting- or growth-stage immediately following secondary spermatogonial mitosis. The ordinary chromosomes have become resolved into an apparent reticulum, in

which their individuality is lost: the heterotropic chromosome is seen in its characteristic form as a darkly staining body apposed to the nuclear membrane. The nucleus is here at its smallest volume.

Fig. 7. The same at a later stage. The growth of the nucleus is very noticeable.

Fig. 8 Beginning of primary spermatocyte prophase. The reticulum has become converted into a highly convoluted spireme, the heterotropic chromosome remaining as a homogeneous mass apposed to the nuclear wall.

Fig. 9. Later prophase of primary spermatocyte. The spireme has broken into filaments, which have become more darkly stained by the closer association of their component granules. These filaments are shortening and thickening and have begun to assume the characteristic ring and boomerang shapes. A nucleolus is shown above the heterotropic chromosome.

## Plate 2.

Figs. 10–19. Chromatin filaments twisted into crosses, rings, and single or double loops; they have become shorter and more condensed than they appeared in the last figure, and will shortly transform themselves into the smooth and compact chromosomes of the metaphase complex.

Fig. 20. Polar view of the primary spermatocyte metaphase—the first maturation division,—showing the nine chromosomes, again divisible into large, small, and medium-sized chromosomes. The heterotropic is still the fourth largest and is marked with a cross.

Fig. 21. Lateral view of the primary spermatocyte metaphase. The ordinary chromosomes are arranged on the mitotic spindle, and the heterotropic chromosome has already passed to the lower pole.

Fig. 22. Ditto.

Fig. 23. Lateral view of the later anaphase of the primary spermatocyte division. The ordinary chromosomes have begun to move towards the opposite poles, but are still attached to one another by the connecting fibrils. The heterotropic chromosome has passed to the upper pole without division. All the chromosomes are shown.

Fig. 24. Lateral view of the primary spermatocyte telophase, showing the massing of chromosomes at the two poles of the spindle.

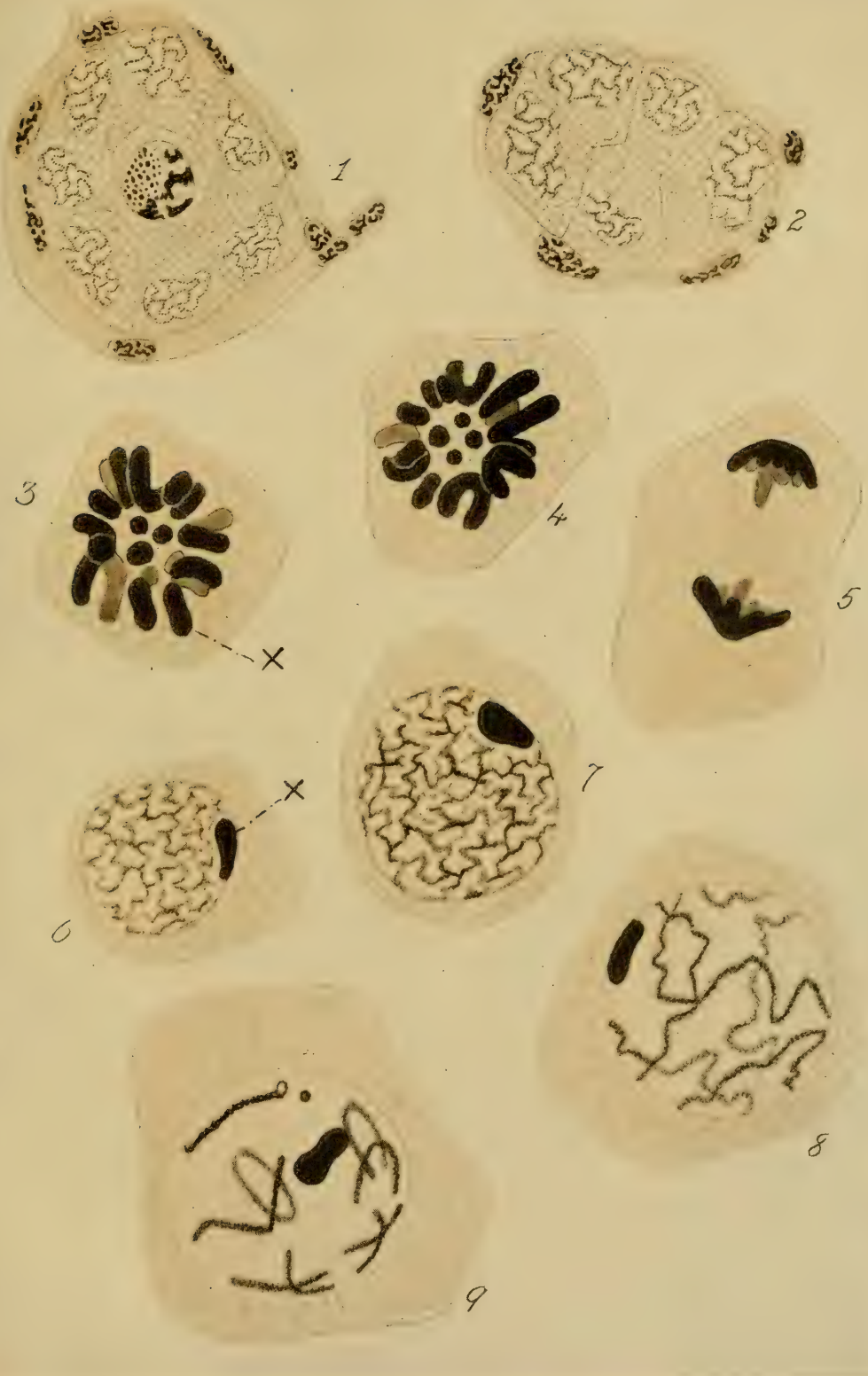
Fig. 25. Polar aspect of the secondary spermatocyte metaphase. The nine chromosomes are exhibited, and are again divisible into three small, three large, two medium, and the odd heterotropic chromosome. Two of the small chromosomes are spherical and the third ovoid; the remainder are seen as a pair of arms joined at one extremity and closely apposed to one another.

Figs. 26, 27. Ditto. It must not be forgotten that the heterotropic chromosome only occurs in 50 % of these cells.

## Plate 3.

Fig. 28. Lateral view of the secondary spermatocyte mitosis—the second maturation division. The heterotropic chromosome is seen on the spindle, with its halves attached to one another by the connecting fibrils, thus appearing as the "lagging" chromosome. The ordinary chromosomes are assembled at the two poles.

Fig. 29. Resting-stage of the spermatid. The ordinary chromosomes have become resolved into their component chromatin particles and have lost their identities; the heterotropic chromosome has become ragged through the same process, but can still be recognized. It is marked X.

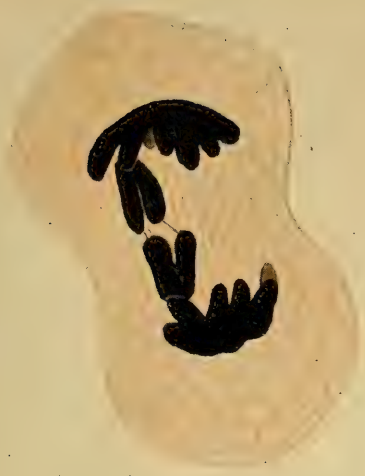


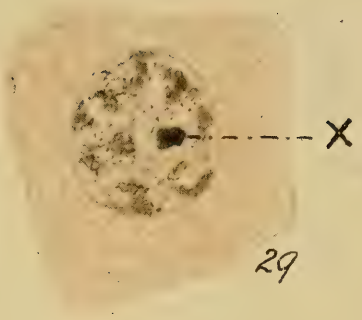

SPERMATOGENESIS IN STENOBOTHRUS.

Grout sc. & imp.

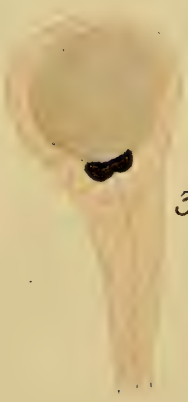

SPERMATOGENESIS IN STENOBOTHRUS.

Grout sc. & imp.

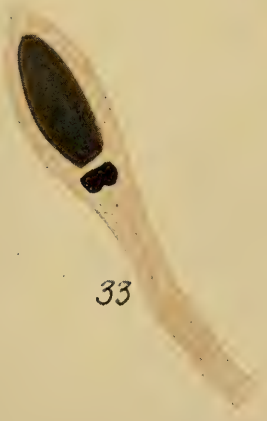


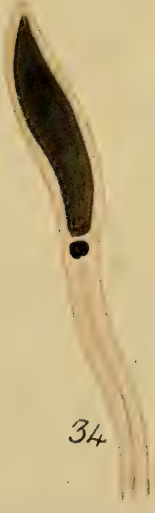














SPERMATOGENESIS IN STENOBOTHRUS.

Grout sc. & imp.

Fig. 30. Later stage of spermatid. The "centrosome" has appeared, and the axial filament is growing out near its constriction. The cytoplasm below the "centrosome" has begun to elongate into the tail.

Fig. 31. Ditto. In this later stage the chromatin particles of the nucleus have become so minute that their individuality is lost.

Fig. 32. A later stage of the above. The nucleus is elongating and is becoming darker. The reduction in size of the "centrosome" is noticeable.

Fig. 33. Ditto.

Fig. 34. The elongation of nucleus and cytoplasm of tail has transformed the spermatid into an unripe spermatozoon. The reduction of the "centrosome" is very marked.

Fig. 35. Ditto.

Fig. 36. Final stage before transformation into the ripe spermatozoon. The darkly stained nucleus is forming the head, the "centrosome" the middle piece, and the axial filament with its surrounding cytoplasm the tail. The "centrosome" has become considerably smaller than it appeared in fig. 35.